

Stem Morphogenesis in *Lycopersicum*: A Quantitative
Study of Cell Size and Number in the Tomato

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Stem Morphogenesis in *Lycopersicum*: A Quantitative Study of Cell Size and Number in the Tomato

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(WITH PLATE 8 AND TEN FIGURES)

In a typical dicotyledenous herbaceous plant, the stem increases in length as the result of the activity of a group of meristematic cells situated at its tip. By these cells the primary tissues are laid down and the fundamental anatomical arrangement of the stem is determined. For several internodes below this meristem, growth takes place in the primary tissues at different rates with the result that the relative sizes of the tissues are changed (Sinnott, 1936). Thus the anatomical pattern is altered. The purpose of this study is to determine what changes take place in the development of the stem in several varieties of *Lycopersicum esculentum* and one variety of *L. pimpinellifolium*, and to discover the mechanism through which the differences in stem size which exist in these types and their hybrids become established.

One of Mendel's original seven characters in peas was concerned with length of stem. Dwarf plants were recessive to tall, and segregated in the F_2 in the ratio of one dwarf to three tall. The inheritance of height in peas was found to be more complicated by Keeble and Pellew (1910) who found two factor pairs concerned with length and thickness of stem respectively. These combined to make a very tall plant. The double recessive produced an extreme dwarf type, and either factor alone produced a plant which was intermediate between the two extremes. Numerous examples of genetic control of height in plants might be cited. In tomatoes the dwarf habit of growth is recessive to the tall, and is controlled by a single pair of Mendelian factors. Thus we have direct evidence that length of stem and height of plant are genetically controlled not only in other plants but in the tomato itself.

The work of Sinnott, Blakeslee and Houghtaling (1934) on *Datura* peduncles offers an excellent example of the anatomical structure of the plant being subject to genetic control. In this genus the presence of an extra chromosome affects both anatomical structure and cell size. Except within the polyploid series, there is little evidence for a necessary relationship between anatomical pattern and size of stem. Large stems may be large through increase in number of cells or through increase in size of cells. Sometimes vascular tissue may be increased without the increase necessarily being accompanied by any other structural enlargement.

However, work on *Datura* was all done at the midpoint of the flower peduncle on the day of flowering. Work has been done on the developmental relationship of cell size to fruit size (Houghtaling, 1935; Sinnott, 1939), and it has been found that, in general, a period of cell division with perhaps some increase in cell size is followed by a period of cell enlargement with no further division. This present study deals with cell and tissue relationships in the development of the main stem of the tomato.

MATERIAL AND METHODS

The material used was derived from three pure lines of *Lycopersicum esculentum*, "Bonnie Best," "Red Cherry," and "Dwarf Champion," and one pure line of *L. pimpinellifolium*, "Red Currant." These had all been inbred through five to eight generations and showed no recognizable genetical variability. Crosses were made between Bonnie Best and Red Cherry, Bonnie Best and Red Currant, and Dwarf Champion and Red Currant. One F_2 of one hundred plants was grown from the F_1 of the cross Bonnie Best by Red Currant. All these plants were grown in the University of Michigan Botanical Gardens during the summer of 1935. More were grown in 1936 and 1937, but the material studied was largely taken from the plants raised in 1935. The photographs (plate 8) were taken in 1937, but comparable measurements showed that these plants were essentially similar to those raised in 1935.

These particular types were chosen for study because they were characterized by large differences in thickness of stem (plate 8). The difference between *L. pimpinellifolium* and *L. esculentum* is, as might be expected, the greatest. Although the various types of *L. esculentum* are vegetatively very similar, still an obvious difference exists between Bonnie Best and Dwarf Champion.

Certain gross measurements were made in the field. Stem and internode length were measured with a ruler, and diameters were measured by micrometer calipers. For all length measurements the meristem was taken as the point of reference. A few mature fruits were measured from

Explanation of Plate 8

Branches from typical plants

- | | |
|-----------------|---|
| 1 = Bonnie Best | 4 = Dwarf Champion |
| 2 = Red Cherry | 5 = Red Cherry $\times$ Bonnie Best |
| 3 = Red Currant | 6 = Dwarf Champion $\times$ Red Currant |



HOUGHTALING: STEM MORPHOGENESIS

every plant. The fruits were cut along a polar axis, and the polar and equatorial diameters were measured with a ruler.

For anatomical study, transverse and longitudinal hand sections were made along the stem at various distances from the meristem. These were stained in safranin and mounted for study in glycerin. For more careful study, material was fixed in seventy percent alcohol, with five percent

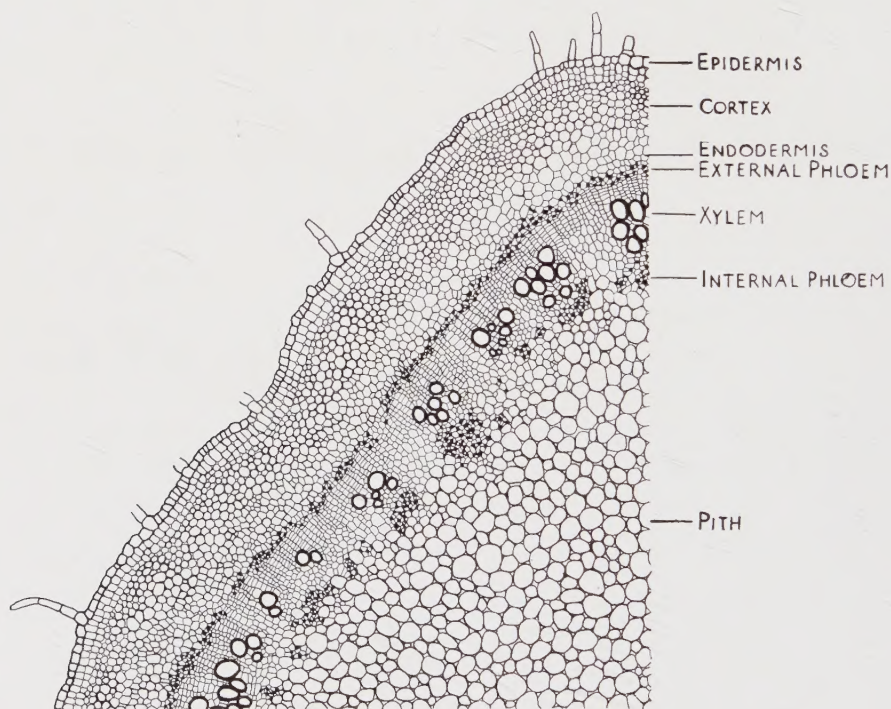


Fig. 1. Diagram of a portion of transverse section through a stem of Bonnie Best showing tissues studied.

acetic acid and five percent formalin added, then imbedded in paraffin, sectioned with a rotary microtome ten to fifty micra thick, and stained in Delafield's haematoxylin and safranin.

The anatomy of the typical tomato stem has been described by Woodcock (1936). Externally the stem is protected by a single epidermal layer (fig. 1). Underneath this is a hypodermal layer which in young stems is one cell thick. This single layer of cells apparently divides, forming two or three layers in older stems. The outermost cells frequently elongate radially. These cells contain chloroplasts. Beneath the hypodermis there is a region of parenchymatous cells which form

the major part of the cortex. An endodermal layer is marked by the presence of starch grains when fresh material is stained with iodine. Woodcock was able to discern Casparian strips, but the author could find none in this material. The cross section of the vascular bundle shows both internal and external phloem, lying in patches on either side of the xylem. The cambium is easily visible, and in older stems considerable secondary growth is evident. Within the ring of vascular tissue lies the pith made up of large parenchymatous cells.

Anatomical measurements were made in various ways, some with a micrometer eyepiece in the ordinary compound microscope, but the majority with a projection apparatus. The sections were put in the machine, the magnified image was projected on paper and drawn, and measurements were made on the drawings. Various magnifications were used, care being exercised that all measurements of a given kind were made at the same time. In measuring cells the ten largest were chosen from each tissue. This was done to avoid those which were not cut through the equatorial diameter and to standardize procedure as much as possible. Ten cells were measured from typical regions of the epidermis, cortex, and pith. In cortical and pith cells, two diameters of each cell were measured at right angles to one another and averaged. The average was recorded as representing the diameter of the cell.

To calculate the cross-sectional area of the cell, this diameter was squared and multiplied by the factor $\pi/4m^2$ where m denotes the magnification. This gave the area of a circle with the original diameter. Thus it was possible to correct for shape and magnification with a single mathematical operation. Since the epidermal cells were not circular in shape it was necessary to multiply the two diameters obtained in the original measurements together to obtain the area of a rectangle and then correct for magnification. These figures, circle and rectangle, closely approximate the actual shape, and hence also the area, of the cells concerned. Further, since the variations from these fundamental figures are at random within a tissue, these calculations may safely be used for the purpose of comparison.

To find a representative cell size for each tissue, the geometric mean of the area of the ten cells measured was calculated. The reason for using geometric means rather than ordinary (i.e., arithmetic) averages lies in the fact that these measurements are of growing cells and growth is a geometric process. Likewise, by the method of computation of cross-sectional areas which was used, the geometric mean was more easily

obtained. In any case the geometric mean differs only slightly from the arithmetic mean and seems to give a more accurate picture of size changes.

Tissue measurements were made from the same sections in which cell size was studied. The stem was projected at a lower magnification and drawn. A line was drawn at the outer limit of the epidermis, one at the outer limit of the external phloem, and one at the inner limit of the internal phloem. Two diameters of each were measured. The area of each was calculated from the average diameter in the same manner as area of pith cells was found. From these areas the cross-sectional area of the stem, cortex, vascular cylinder, and pith were computed.

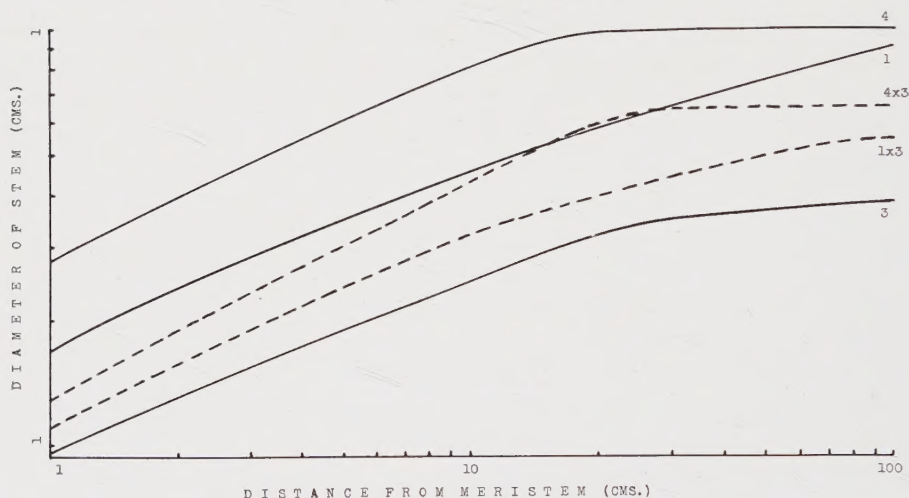


Fig. 2. Diameter of stem plotted logarithmically against distance from the meristem. Each curve represents approximately 100 measurements.

4 = Dwarf Champion

1 = Bonnie Best

4 × 3 = Dwarf Champion × Red Currant

1 × 3 = Bonnie Best × Red Currant

RESULTS

The differences in diameter of stem and length of internodes are shown in the photographs (plate 8). Measurements show that at equal distances from the stem tip these various tomato varieties have marked differences in diameter. Figure 2 shows the diameter of the stem measured at the mid-point of the internode plotted logarithmically against the distance of that point from the tip of the stem. In the pure lines the difference in stem diameter is already established at one centimeter from the tip. Measurements with calipers were not very accurate within a distance of one centimeter from the tip because of the proximity of young leaves. However,

examination of microscope sections shows that the differences in stem size continue back into the meristem itself. The curves in figure 2 show that the differences which are established so early are maintained throughout the growth of the stem. For example, at one centimeter from the tip the stem diameter of Bonnie Best is a little less than twice that of Red Currant, at five centimeters from the tip the former is still just a little less than double the latter, and at ten centimeters the same relationship holds. In other words, relative increase in stem diameter takes place at the same rate with respect to distance from the meristem in both types. Figure 2 shows that this is true for all the types studied. The tendency for the curves to flatten between ten and twenty centimeters from the tip is undoubtedly because of the termination of increase in diameter by primary growth with the assumption of secondary growth. The hybrid, Bonnie Best $\times$ Red Currant, lies between its two parents, rather nearer Red Currant.

Table 1 gives the average diameters of the stems at five and ten centimeters from the tip. For the hybrids, the arithmetic and geometric means of the parents are given for comparison with the attained values. It is obvious that the hybrids lie very close to the geometric means. The fact that the values obtained from the measurements are slightly smaller in all

TABLE 1
Diameters of Stems

LINE	DIAMETER 5 CMS. FROM TIP	DIAMETER 10 CMS. FROM TIP
RED CURRANT	.187	.250
BONNIE BEST	.350	.450
DWARF CHAMPION	.595	.800
BONNIE BEST $\times$ RED CURRANT	.240	.325
<i>Arithmetic mean</i>	.265	.350
<i>Geometric mean</i>	.256	.335
DWARF CHAMPION $\times$ RED CURRANT	.300	.425
<i>Arithmetic mean</i>	.386	.525
<i>Geometric mean</i>	.324	.447

instances suggests partial dominance of the smaller parent. The slightly steeper slope of the hybrid, Dwarf Champion $\times$ Red Currant, indicating more rapid relative increase in stem diameter, may be the result of hybrid vigor, although there is no indication of the same effect in the other hybrid.

In the course of this work the author noted that in the F_2 population, in which there was complicated segregation of fruit size (fig. 3), the plants

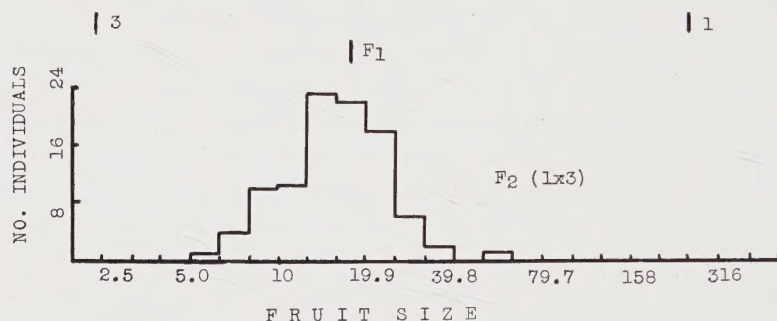


Fig. 3. Diagram shows distribution of fruit size on a logarithmic scale in the F_2 from a cross of Bonnie Best $\times$ Red Currant. The positions of the mean value of fruit size in the parents and F_1 are indicated by vertical lines.

with the thicker stems bore larger fruits than those with thinner stems. In the parental types this relation is fairly obvious. The stem diameter of Red Currant at ten centimeters from the tip is .250 cms., and the fruit

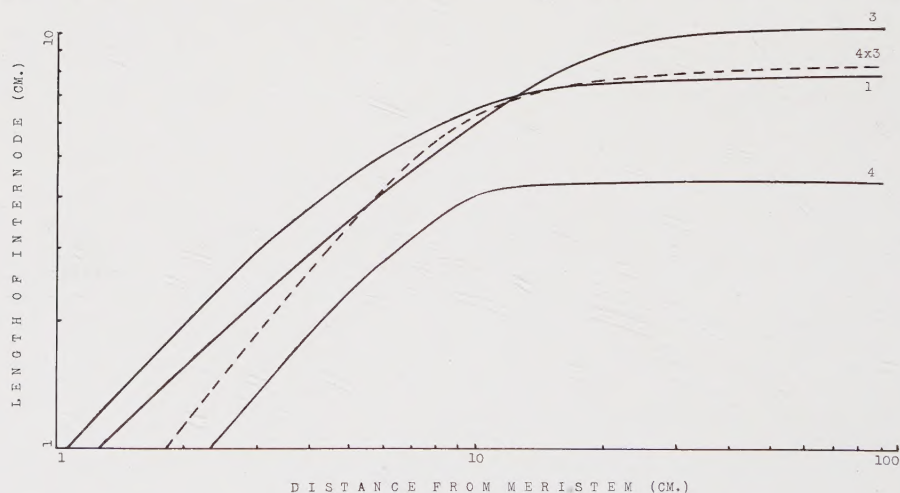


Fig. 4. Length of internodes plotted logarithmically against distance from the meristem. Each curve represents approximately 100 measurements.

3 = Red Currant

1 = Bonnie Best

4 = Dwarf Champion

4 $\times$ 3 = Dwarf Champion $\times$ Red Currant

volume is about 1.5 cu. cm. The corresponding figures for Bonnie Best are .450 cm. for the diameter at ten centimeters and 275 cu. cm. In the F_2 from the cross Bonnie Best $\times$ Red Currant, a correlation of $.57 \pm .03$

was found between size of fruit and diameter of stem measured ten centimeters from the meristem. This relation may, of course, be the result of genetic linkage between factors controlling stem and fruit size. It may also be physiological in that small-stemmed types may be unable to bear large fruits. However, since the diameter of the stem is determined in the meristem and Houghtaling (1935) has shown that the size of the fruit is also determined in the fruit meristem, it is suggested that a morphological correlation may exist between the meristems of the plant.

The illustrations (plate 8) indicate little difference in internode length except in Dwarf Champion. Statistical data show that this length is subject to great variability. There seem to be no or only slight differences existing among all the types with the exception of Dwarf Champion (fig. 4). In the latter the internodes are relatively shorter than in the other varieties, not only at first but throughout development so that the relative internode length is maintained. It seems rather surprising that with the great difference in diameter that exists between Bonnie Best and Red Currant there should be practically no difference in length of internodes. The factors which control diameter have no influence upon length.

Another point of some interest which appeared in measuring internode length is the fact that the internode below a branch is generally shorter than the internode above the branch although the latter is, of course, the younger. This is particularly noticeable in the thick-stemmed types. For example, in Bonnie Best the internode below a branch averages 67 percent as large as the internode above the branch; in Dwarf Champion, 79 percent; in Red Cherry, 88 percent; and in Red Currant, 89 percent.

In figure 5 the relation is shown between cross-sectional area of the tissues in the stem and the area of the stem. The distances between the lines indicate the differences in cross-sectional area of a single tissue in the several varieties. The slope of the lines indicate the relative rate of growth of the tissues with respect to stem area, or, more simply, the value of Huxley's constant, "k" (Huxley, 1932).

For the pith the slope of the lines, or "k," is always unity, which indicates that the area of the pith increases at the same rate as the area of the stem. In other words, the area of the pith occupies the same proportion of the stem throughout development. The differences in position of the lines show that relatively more of the stem is made up of pith in Dwarf Champion than in Bonnie Best, and more in Bonnie Best than in Red Currant. The hybrid between these last two lies intermediate between them.

The fact that these lines do not converge at the base indicates that the differences in size of pith are initial differences determined in the meristem and maintained as such through development. It is interesting to note (cf. table 2) that the area of the pith in the types with larger stems is proportionately greater than in those with smaller stems.

For cross-sectional area of vascular tissue the story is not quite so simple. The slopes of the lines are different, being greater for Red Currant than for Bonnie Best, with the hybrid lying between. Dwarf Champion has the least slope of all. This is, of course, in the reverse order of stem size. There is a tendency for the lines to converge at the base. Thus if Dwarf Champion ever produced a stem as small as the smallest studied of Red Currant it would have approximately the same amount of vascular tissue. However, the rate of increase of vascular tissue in Red Currant is much greater than in Dwarf Champion with the result that ultimately the small-stemmed type has a much larger proportion of vascular tissue. The rate of increase of cross-sectional area of vascular tissue with refer-

TABLE 2
Cross sectional areas of Stems and Huxley's Constant, k

LINE	CROSS-SECTIONAL AREA OF STEM AT 10 CMS.	PERCENT PITH	K VASC. CYL. AND STEM	K CORTEX AND STEM
RED CURRANT	.0492	30	1.26	.77
BONNIE BEST	.1580	40.5	1.10	.84
DWARF CHAMPION	.5120	49	1.05	.87
BONNIE BEST $\times$ RED CURRANT	.0860	39	1.14	.80

ence to cross-sectional area of the stem is inversely proportional to the typical stem thickness of the varieties studied (table 2).

In the pith the slope of the lines for all types is 1. In the vascular cylinder the slope, or "k," is always greater than 1. The area of the vascular tissue in the cross-section increases faster than the stem as a whole. Therefore the cortex cannot increase as fast as the area of the

Explanation of Figure 5

Fig. 5. Cross-sectional area of cortex, vascular cylinder, and pith plotted logarithmically against cross-sectional area of stem.

	NO. OF SECTIONS
1 = Bonnie Best	32
4 = Dwarf Champion	17
3 = Red Currant	34
1 $\times$ 3 = Bonnie Best $\times$ Red Currant	23

The curve showing the relation of the cortex to the stem area for the hybrid was omitted because it lay too close to the curve for Red Currant to show distinctly.

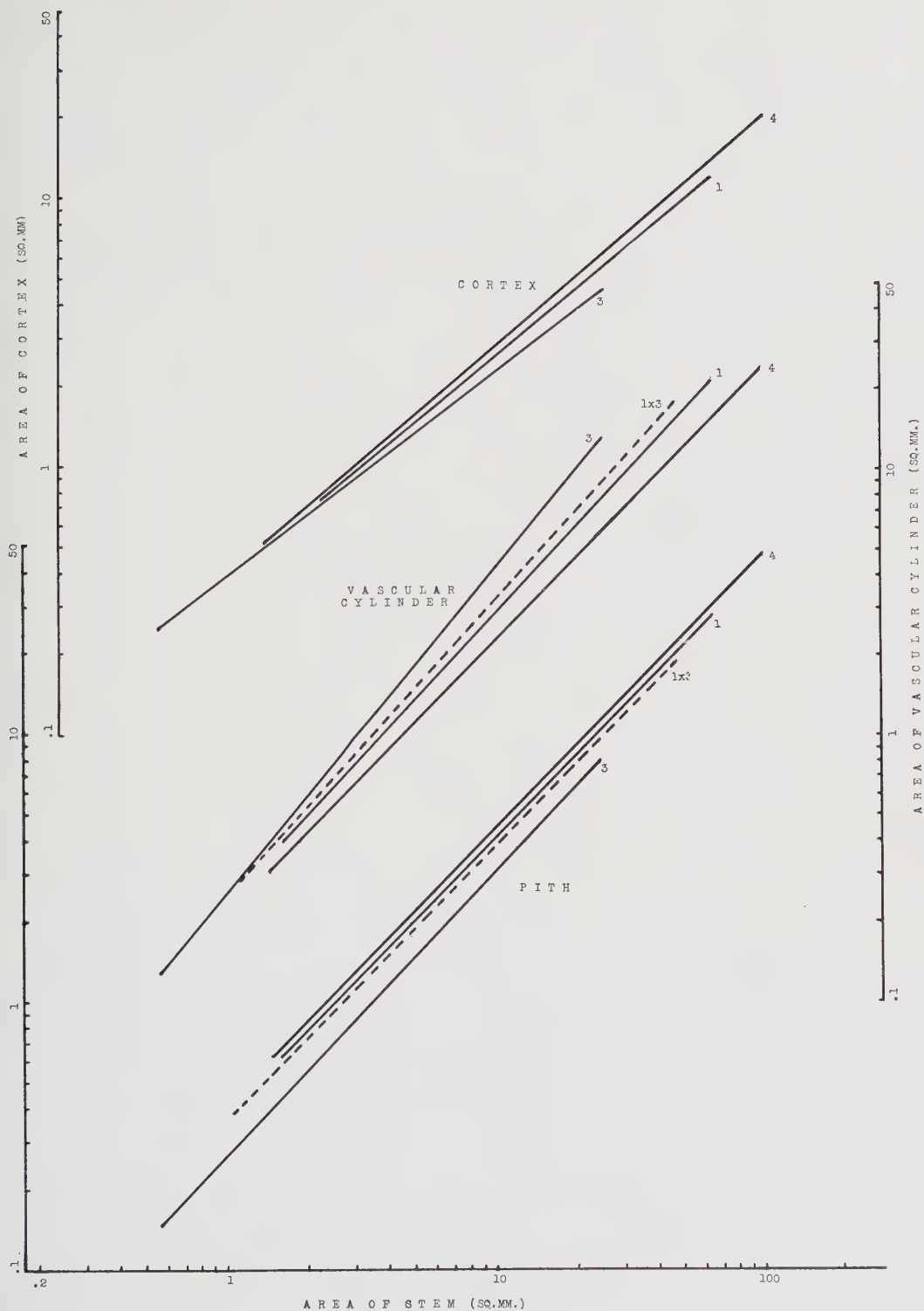


Figure 5

stem. Also since the greatest rate of increase of vascular tissue is found in Red Currant, it is obvious that the slowest rate of increase in the cortex must be found in Red Currant. The lines in figure 5 and the values of "k" for relative rate of increase of cortex with respect to stem in table 2 show that this expectation is fulfilled. Whereas the rate of increase of

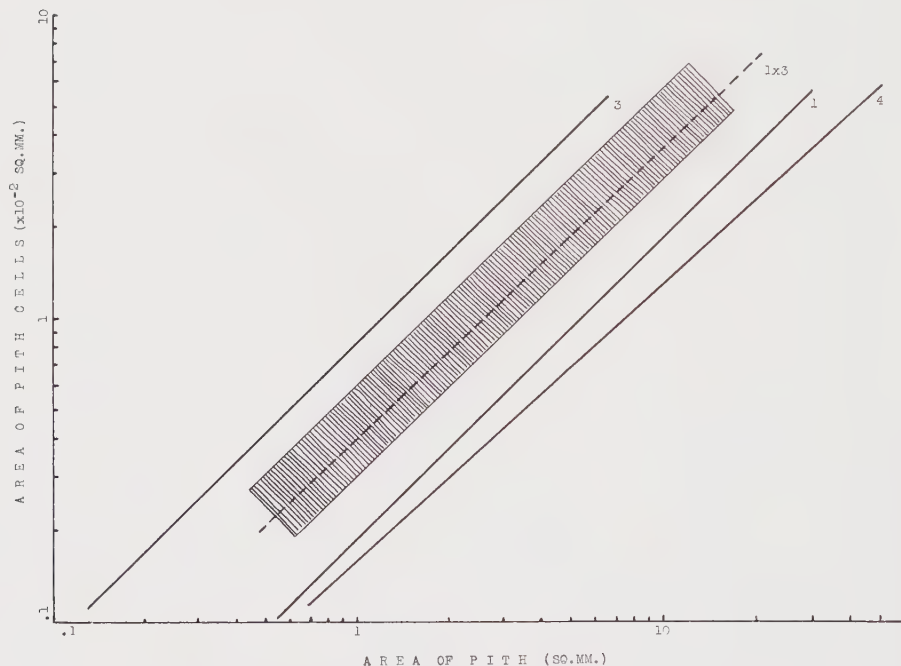


Fig. 6. Cross-sectional area of pith cells plotted logarithmically against cross-sectional area of pith. The shaded area shows the range of the F_2 curves.

	NO. OF CELLS MEASURED
4 = Dwarf Champion	170
1 = Bonnie Best	320
3 = Red Currant	340
1 $\times$ 3 = Bonnie Best $\times$ Red Currant	170

vascular tissue increases inversely with the stem size of the various types, the rate of increase of cortical tissue varies directly with the stem size.

Cell size was compared with tissue size. Figure 6 shows the relation between cross-sectional area of pith cells with cross-sectional area of pith. The slope of the lines, or "k," is approximately 1, and the cells increase in cross-sectional area at the same rate as the pith. Evidently cell division has stopped and increase in pith area is accomplished by increase in cell size. This is true in all types studied. However, for a pith of given size,

large differences in cell size appear. Red Currant, the smallest-stemmed type, has the largest pith cells. Dwarf Champion, the largest-stemmed type, has the smallest cells, and the other kinds are intermediate in order of their stem size. Figure 7 shows that the same condition holds for cortical cells. The differences in cell size, both initial and ultimate, are smaller than in the pith, however.

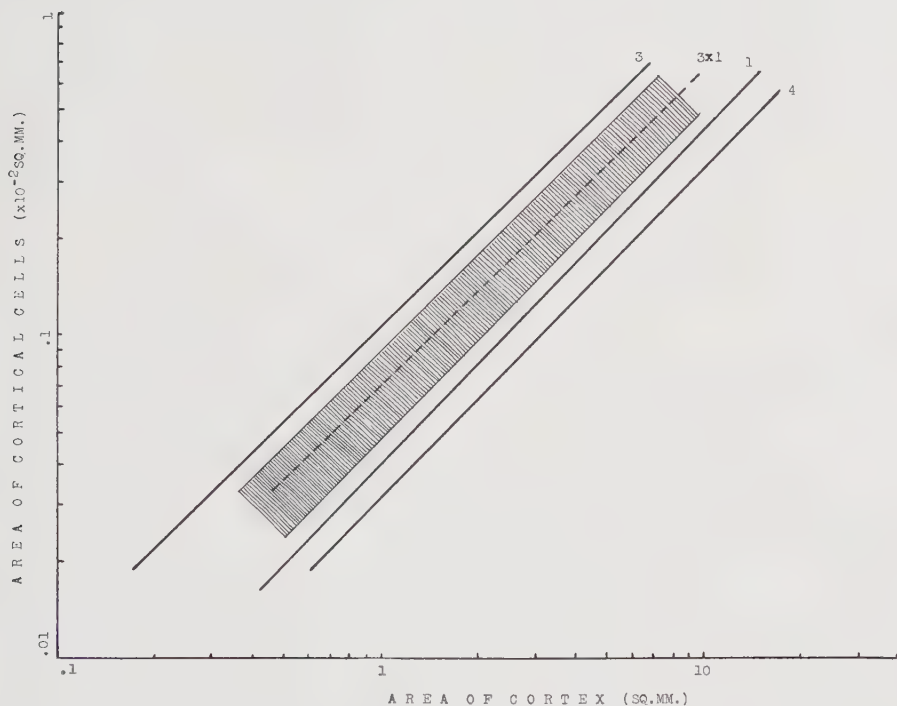


Fig. 7. Cross-sectional area of cortical cells plotted logarithmically against cross-sectional area of cortex. The shaded area shows the range of the F_2 curves.

	NO. OF CELLS MEASURED
4 = Dwarf Champion	170
1 = Bonnie Best	320
3 = Red Currant	340
1 $\times$ 3 = Bonnie Best $\times$ Red Currant	230

The cross-sectional area of the epidermal cells is compared with the cross-sectional area of the stem (fig. 8). This relation is probably not a causal one, but it does indicate that the size of the cells does not increase as rapidly at first as the size of the stems, which means that for some time cell division continues to occur. Later the slope of the curve does approach 1, at which time cell division has stopped. It has long been known (Brotherton and Bartlett, 1918; Sinnott, 1930) that cell division may persist

longer in the epidermal cells than in the other primary tissues. Most interesting is the fact that the order of the curves shows that the same relative cell size and tissue or stem relationship holds here that exists in the other tissues. Red Currant, the smallest-stemmed type, has comparatively the largest cells in all tissues, with the hybrid Bonnie Best $\times$ Red Currant next, then Bonnie Best, and finally Dwarf Champion. Thus the larger the stem of a variety, the smaller its cells are with respect to those of the other varieties.

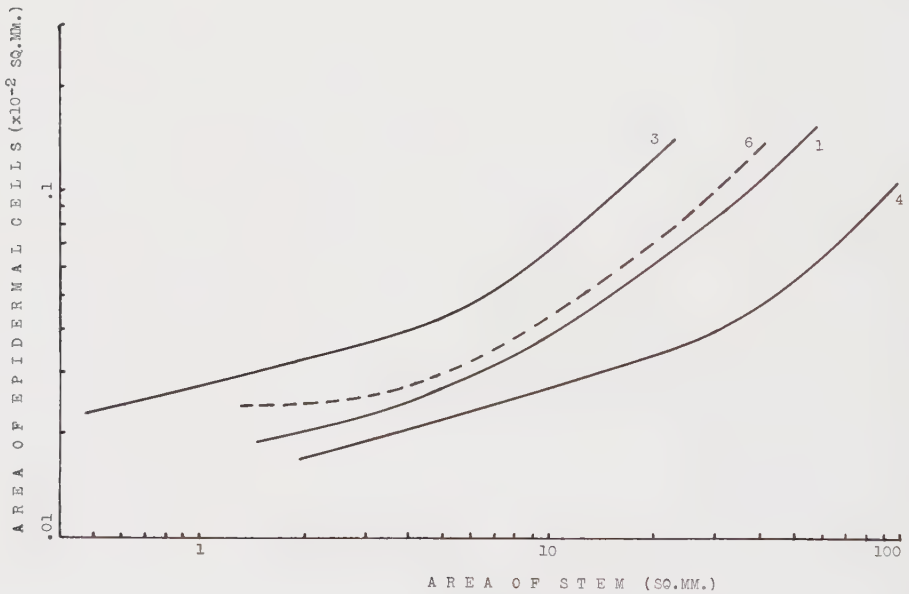


Fig. 8. Cross-sectional area of epidermal cells plotted logarithmically against cross-sectional area of stem. The number of cells measured for each curve varies from 170 to 370.

3 = Red Currant
1 = Bonnie Best

4 = Dwarf Champion
6 = Dwarf Champion $\times$ Red Currant

In a pith with cross-sectional area of one square millimeter, Red Currant will have much larger cells than Bonnie Best will have in pith of equal size. But Bonnie Best starts development with a much larger pith than Red Currant and therefore pith area of one square millimeter will be found in much younger stem of Bonnie Best than in Red Currant. Thus the possibility of a relationship between cell size and age, or, perhaps more suitably, degree of maturation, is suggested. The degree of maturation, a function of time, can be obtained only through some indirect measurement. The measure which suggested itself was the distance from the

meristem. Figure 9 shows the relation to distance from the meristem of stem diameter, pith diameter, thickness of vascular tissue, and diameter of pith cells, for one individual stem from a plant each of Red Currant and Bonnie Best. These are all plotted on a logarithmic scale to the base

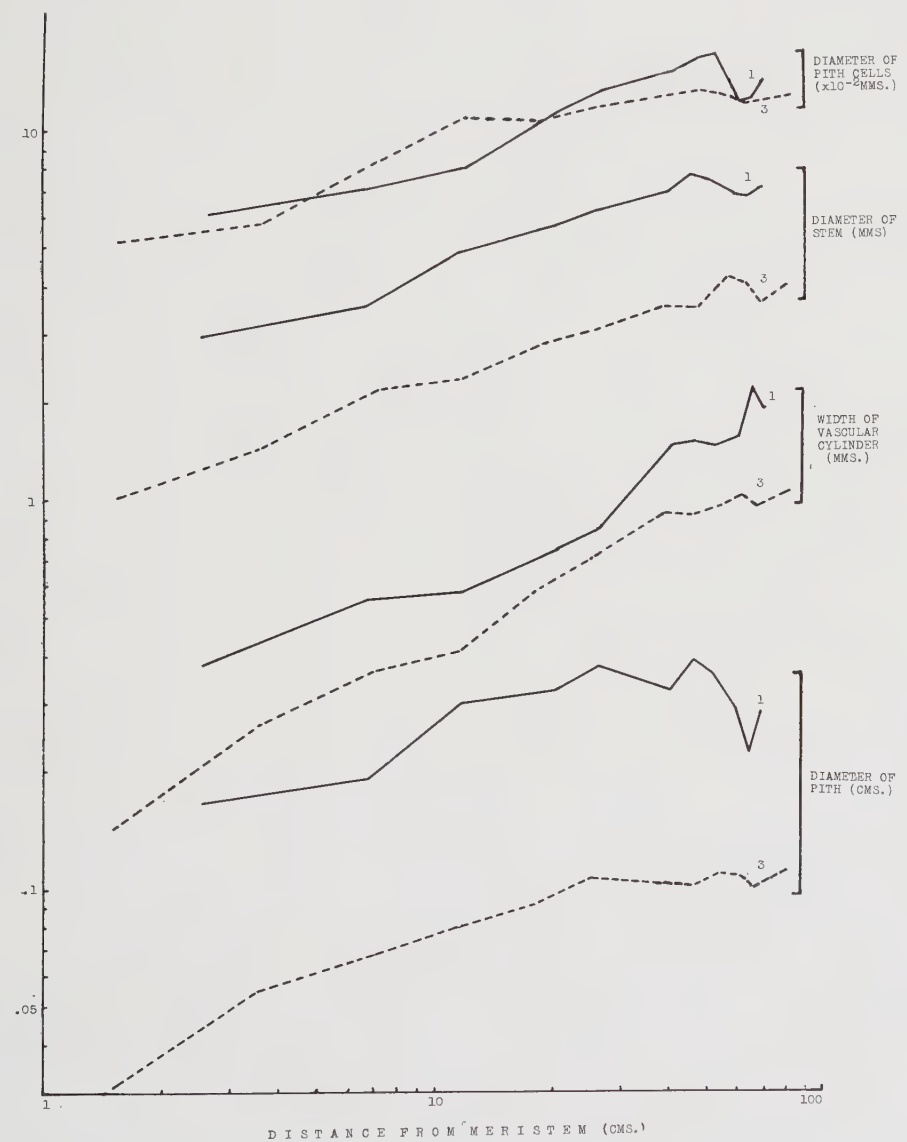


Fig. 9. Diameter of pith cells, stem, pith, and width of vascular tissue plotted logarithmically against distance from meristem.

1 = Bonnie Best

3 = Red Currant

ten with equal intervals representing the multiple ten (as, for instance, those from 1 to 10 and from 10 to 100, respectively). Thus the distances between the curves for Bonnie Best and Red Currant for stem diameter may be compared directly with the distances between the curves for pith cells.

With this in mind it is obvious from the figure that although great differences in size of pith exist, there is no difference in pith cell size among the varieties with long internodes if distance from the meristem is the same. Also, the size of cortical cells is exactly parallel, and is strictly a function of distance from the meristem. This is further brought out in table 3 where cell size is given at various intervals down the stem. There is very close agreement for Bonnie Best, Red Currant, and their hybrid.

TABLE 3
Cross-sectional Area of Cells

LINE	DISTANCE FROM MERISTEM							
	1 cm.		2.5 cm.		5 cm.		10 cm.	
	Pith	Cortex	Pith	Cortex	Pith	Cortex	Pith	Cortex
BONNIE BEST	.160	.0295	.39	.062	.71	.104	1.20	.167
RED CURRANT	.165	.0359	.37	.062	.74	.103	1.27	.162
BONNIE BEST × RED CURRANT..	.180	.0375	.37	.060	.88	.100	1.32	.165
DWARF CHAMPION	.485	.0620	1.12	.137	1.95	.270	3.60	.400

Bonnie Best and Red Currant are classified as belonging to different species; the former, *Lycopersicum esculentum*, and the latter, *L. pimpinellifolium*. Lindstrom and Humphrey (1933) found that *L. pimpinellifolium* has smaller chromosomes (although the number is the same) than *L. esculentum*. Navashin (1931) working on *Crepis* found a direct relation between amount of chromosomal material and cell size in the meristem. In these two species of tomato, cells were measured in longitudinal sections of the meristem with an eyepiece micrometer and no difference was found in area. The method of making measurements was not as accurate as that employed by Navashin, and it is possible that a more refined technique would show differences. However, in view of the results in older tissues, differences do not seem probable.

From table 3 it is apparent that the cells in the pith (or in the cortex) of Bonnie Best and Red Currant and their hybrid agree almost exactly in size at equal distances from the tip of the stem. This is not true in Dwarf Champion in which the cells are very much larger. In Bonnie Best and

Red Currant internode length was the same, but in Dwarf Champion the internodes were shorter. It was thought that perhaps at an equivalent number of internodes from the tip cell size in Dwarf Champion would be the same as in the other types. In making measurements the number of internodes was not counted. However, since it was known that the internodes in Bonnie Best are approximately twice as long as in Dwarf Champion, it might be assumed that at 2.5 centimeters from the tip Dwarf Champion would have as many internodes as Bonnie Best at 5 centimeters from the tip, etc. It is obvious from the table that the cells in Dwarf Champion are still much larger than would be expected on this assumption. However, length from the tip in the morphologically similar varieties and their hybrid was conceived to be an indirect measure of a time interval, and there is no evidence that number of internodes in morphologically different varieties can be treated as a measure of time any more than distance from the meristem.

It has already been pointed out that cell size in the pith and in the cortex, as measured by cross-section, increased at the same rate as tissue. This would indicate that no cell division occurred in these tissues below the meristem. In order to check this conclusion, the cross-sectional area of the tissue was divided by the area of the cells and thus cell number was computed. Although there was considerable variability within each variety, there was no evidence that such variability was not completely at random. Attempts to correlate cell number with tissue cross-section within a variety showed that no relation existed at all and the conclusion was definitely reached that cell number within a variety does not increase below the meristem.

The individual plants of the F_2 from the cross Bonnie Best $\times$ Red Currant were examined developmentally in the same way that the parents were. Fundamentally the same relations held. Cell cross-section increased at the same rate that tissue cross-section did, etc. The group of curves (See figs. 6 and 7), each representing a single individual, fell between the curves for the parents. As might be expected they showed a wider spread than equivalent curves for the parents or F_1 , indicating greater variability and thus, as usually interpreted, multiple factor inheritance. There was no clear-cut segregation. These curves were rather difficult to deal with inasmuch as the variability in one individual frequently threw it into the size class of individuals on either side. A large number of measurements would be necessary to locate the curves accurately, and the additional information which would be obtained did not seem sufficient to war-

rant the effort. However, since cell number is fairly constant in the various cross-sections of the individual, it offered a fairly simple means of comparing parents and offspring for that characteristic. The results are given in figure 10 and in greater detail in table 4.

TABLE 4
Comparison of Cell Numbers of Parents and Offspring

		PITH CELLS		CORTICAL CELLS		NUMBER OF CELLS MEASURED
		Number	Standard deviation	Number	Standard deviation	
PURE LINES	BONNIE BEST	563	34	2430	162	320
	RED CHERRY	450	20	2070	169	370
	RED CURRANT	127	5	1045	66	340
	DWARF CHAMPION	664	40	2910	107	170
F ₁	RED CHERRY × BONNIE BEST	425	30	1725	128	200
	BONNIE BEST × RED CURRANT	248	14	1410	67	230
F ₂ : BONNIE BEST × RED CURRANT	1	187	13	1060	88	
	3	193	12	1210	121	
	5	149	10	1160	106	
	6	187	15	1290	93	
	9	146	8	1210	121	
	13	219	15	1530	218	
	14	178	15	1260	87	
	15	165	13	943	124	120
	16	252	18	1690	191	to
	17	198	9	1210	104	200
	18	203	17	1020	77	from
	19	204	17	1205	205	each
	20	213	16	1050	72	plant
	64	304	36	1970	163	
	67	220	17	1210	171	
	68	219	9	1405	136	
	69	280	11	1820	75	
	70	219	14	1585	169	
	71	323	22	2060	280	
	72	262	14	1680	139	
	73	345	21	2105	160	

Standard errors are given in the table for the calculated values of cell number for each plant. It is obvious that real differences in cell number exist in both cortex and pith. Also it appears that cell number in the cortex is related to cell number in the pith. The value of the correlation is $.653 \pm .125$. Further, within the F₂, cell number in the pith was related to the diameter of the stem at a distance of ten centimeters from the meristem. The value of the correlation was found to be $.587 \pm .182$, in-

dicating that stem size and cell number are related. This relation also holds in the parents. Although in this F_2 the relation may be due to genetic linkage, it seems more plausible to assume that it is a structural necessity, and that the larger stems are larger through the possession of more cells than the smaller. This is of particular interest since it indicates that genetic differences may depend directly upon cell number.

DISCUSSION

Sinnott (1936) studied the relations between the primary tissues in a series of young shoots from a tree of *Pinus Strobus*, in a group of flower stalks of *Datura Stramonium*, and in a series of steles of the fern *Todea*

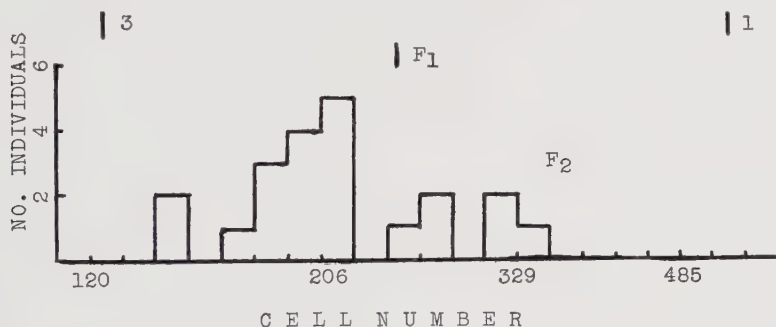


Fig. 10. Diagram shows the distribution of pith cell number in the F_2 of the cross between Bonnie Best and Red Currant. The positions of the mean values of the parents are indicated by vertical lines.

hymenophylloides. Considerable differences in stem size existed within these groups. He found in all of these that the diameter of the pith increased very much faster than the diameter of the stem, the diameter of the vascular cylinder slightly faster or at the same rate as the stem, and the diameter of the cortex very much more slowly than the diameter of the stem. He suggested several possible explanations for the position of the vascular cylinder; first, that it was determined by the position of the leaf primordia; then, that the vascular cylinder may be differentiated at the place where the two different growth rates of pith and cortex come together in the meristem; and lastly, that it is a matter of genetically determined pattern. He says (page 421), "No simple physiological explanation presents itself for these changes in relative size with increasing absolute size. This may be an expression of a developmental pattern inherent in the constitution of the organism."

Buchholz (1938) examined the relationships of tissue size in stems of different dimensions collected from branches of old trees of *Sequoia gigantea*. He found that diameter of stele, diameter of wood cylinder, and diameter of pith plotted against diameter of stem gave a value for "k" of 1.7. Thus pith and vascular tissue were growing at the same rate and very much faster than the stem. Although no figures are given for the cortical tissue it must have grown much more slowly than the stem. He also examined stems which bore male and female cones. The diameter of the stems bearing female cones was very much greater than those bearing male cones. In both, the cone-bearing branches had a relatively smaller pith and larger vascular cylinder than comparable vegetative stems. He found that large stem tips have broader promeristem points with many more cells than those of small stem tips. He presents a very interesting diagram of the promeristems from which the stems of the various types and sizes must have originated. He places all size differences as having their origin in the promeristem.

The two papers just cited both deal with stems which differ in size but not in age. This present paper, on the other hand, deals with size differences which at different levels within the individual plant are correlated with differences in age. Individual plants within a "pure" (i.e., inbred) line are so nearly identical that parts of one may be substituted for exactly corresponding parts of another. The differences among the "pure" lines themselves (the inbred varieties) and among the individual plants of the F₂ are, of course, genetic in origin. That it is possible to prove striking similarities has been shown in the discussion of cell size in Bonnie Best and Red Currant, whereas extremely different cell and tissue relations were exemplified by the contrast between the two aforementioned varieties and Dwarf Champion.

The developmental study gives results which are not entirely in accordance with those of Sinnott. Thus, the gradient of growth rates found by him does not hold since in all the tomato varieties the vascular tissue grows most rapidly. However, in the latter some secondary growth occurred, although the curve which shows the relation of growth of vascular cylinder to stem tissue remains a straight line, indicating no change in relative growth within the beginning of secondary growth. Aside from this, the difference between rate of growth in cortex and pith holds as in Sinnott's material, although the differences are not as extreme as those found by him.

Within the primary tissues cell number seems to be determined in the meristem. Cell size is directly related to the distance of those cells from the meristem. The fact that the cells in the cortex are much smaller than those in the pith may be related to the slower growth of that tissue. The persistence of cell division in the epidermis may be related to the smaller ultimate size of the cells which may be under functional control. It is generally assumed that pith and cortical cells play a comparatively small rôle in the physiology of the plant. The evidence found here for cell number in general points directly to the conclusion that cell number may be genetically controlled without any influence upon cell size.

Between the "pure" lines (varieties) the differences are largely meristematic in origin. Cell number certainly is, and likewise size of pith. The rate of growth of vascular tissue is different in the different types but there is a regularity in the difference. The smaller the stem the more rapid the growth of vascular tissue. The reason for this may be connected with the physiology of the plant. Although the leaves are smaller on the small-stemmed types, the length of the internodes is the same, with one exception, so that approximately the same number of leaves is produced. Thus transpiration in the thin-stemmed types would not be a great deal less than in the thick-stemmed types. Although the vascular cylinder grows more rapidly there is never actually as much in the small-stemmed as in the large-stemmed types for the number of leaves which it supports. Numerous instances of increase in vascular tissue in response to demand exist in the literature. Of course, in this present case the differences in growth rates of the vascular tissue may be the result of genetic factors, but it seems more plausible to consider it in the nature of a secondary and physiological response.

The reason for the decreasing rate of growth in the cortex is not obvious. Since the growth rate of the cortex is smallest where the growth rate of the vascular tissue is greatest there appears to be a compensatory effect. This suggests that perhaps the functional demand cited in the previous paragraph may determine the distribution of food or growth hormones and so account for these differences in growth rate. This seems to be in contradiction to Sinnott's hypothesis that differences in rate of growth of cortex and pith determine the position of the vascular cylinder. It is difficult to imagine a mechanism of control which would act first on the cortex and secondarily on the vascular tissue since their relative functional importance is reversed. However, if the control is directly genetic such action is possible.

SUMMARY

The development of the stem was studied in three varieties of *Lycopersicum esculentum* and one variety of *L. pimpinellifolium*, in two hybrids between the species, and in one F₂ grown from one of the hybrids.

It was found that the rate of increase in diameter of stem with reference to the distance from the stem tip is the same in all types in spite of large initial and ultimate differences.

The length of internodes at corresponding distances from the meristem in plants of the same age is approximately the same in all types studied except in one variety of *L. esculentum*, Dwarf Champion, in which internode length is less. In general the internode below a branch is shorter than the one above. This branch effect is greatest in the thick-stemmed types.

The rate of growth of the different internal tissues is not the same. In general the pith increases in cross-sectional area at the same rate as the stem; the vascular cylinder enlarges faster and the cortex slower.

In the thinner-stemmed types the rate of growth of the vascular tissue as compared with growth of the stem is more rapid than in the thicker-stemmed types, and the rate of growth of the cortical tissue is correspondingly slower.

Cell cross-sectional area in the pith and in the cortex increases at the same rate as cross-sectional area of pith and cortex.

Epidermal cell number and cell size increase as the area of the stem increases.

Cell size in the cortex and in the pith in all but Dwarf Champion is the same at equal distances from the stem tip.

The differences in size of cortical and pith tissues in the different varieties is the result of differences in cell number. The hybrids lie intermediate between their parents and the positions of the F₂ plants indicate segregation and thus that cell number is inherited. No F₂ plant lies outside the cell number range of the parents.

The anatomical pattern possesses a regularity with relatively slight deviations which suggest a generic rather than a specific mechanism of control. Whereas minor deviations are under control of genetic factorial combinations that are characteristic of the several varieties and species, for *L. pimpinellifolium* has been held to be different specifically from *L. esculentum*, it is evident that for the genus *Lycopersicum* the mechanism involved is the same in all types.

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